Section: Anatomy

Original Research Article

A CADAVERIC STUDY ON THE COMPARISON OF HISTOMORPHOMETRIC PARAMETERS OF PRE-TRANSVERSE SEGMENT OF RIGHT AND LEFT VERTEBRAL ARTERIES

Kanchan Bala Dogra[1], Manjunath V Motagi[2], Yuganti Prabhakar Vaidya[3]

[1]Assistant Professor, Department of Anatomy, Chirayu Medical College and Hospital, Bhopal, Madhya Pradesh, India
[2]Professor & Head, Department of Anatomy, Sri Aurobindo Institute of Medical Sciences, Indore, Madhya Pradesh, India
[3]Professor & Head, Department of Anatomy, Peoples College of Medical Sciences and Research Centre, Bhopal, Madhya Pradesh, India

Received : *20/10/2023*
Received in revised form : *28/11/2023*
Accepted : *07/12/2023*

Keywords:
vertebral artery, histomorphometry, inner diameter, outer diameter.

Corresponding Author:
Dr. Kanchan Bala Dogra,
Email: drkanchandr01@gmail.com

DOI: 10.47009/jamp.2023.5.6.127

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (6); 617-621

Abstract

Background: The vertebral artery (VA) is unique amongst the head and neck vessels because of its smaller ratio of diameter in relation to its length, division into four parts and joining of the bilateral paired arteries into one in the form of basilar artery. It supplies hind brain centers that control cardiac, respiratory and equilibrium functions. The aim is to compare the histo-morphometric parameters such as length, outer diameter, inner diameter, width of tunica intima and tunica media of first part of right and left vertebral arteries. **Materials and Methods:** Pre-transverse segment of 40 right and left vertebral arteries was studied. Length of the segment on both sides was measured using Vernier callipers. Tissue obtained from the dissected vessel was histologically processed and stained with Hematoxylin and Eosin. Inner diameter, outer diameter, width of tunica intima and tunica media were measured using Motic Images Plus software. The observations were tabulated and compared using SPSS version 29. **Result:** The length of V1 segment on right side was 42.51±5.41 and on left side was 42.64±4.89. The outer diameter on right side was observed to be 2.84±0.60 and on left side, it was 3.21±0.63, the difference was statistically significant. Statistically significant differences were observed in inner diameter on right (2.63mm) and left side (2.93mm). Width of tunica intima was 30.01µm on right side and 31.01µm on left side. Width of tunica media was found to be 68.87µm on right and 72.56µm on left side. **Conclusion:** The left sided vertebral artery was found to be dominant.

INTRODUCTION

Vertebral arteries (VA) are possibly, the most pivotal of medium-sized arteries in the body, which supply hind brain centers which are concerned with the control of cardiac, respiratory and equilibrium functions. It has a long course which includes the extracranial and intracranial segments.[1,2] The VA is unique amongst the head and neck vessels because of its smaller ratio of diameter in relation to its length, division into four parts and joining of the bilateral paired arteries into one in the form of basilar artery.[3] Anatomically, VA is divided into 4 segments:

1. V1: The first segment passes backwards and upwards and lies between the longus coli and scalenus anterior muscles and lies posterior to the common carotid artery up to foramen transversarium of 6th cervical vertebra.
2. V2: The second part goes upwards and passes through the transverse foramen of upper six cervical vertebrae.
3. V3: This segment starts from the foramen on the medial side of the rectus capitis lateralis muscle and passes postero-medially behind the lateral mass of atlas and is visible on the groove present on the superior aspect of posterior arch of atlas vertebra and finally enters the vertebral canal.
4. V4: The fourth part is present intracranially and meet the opposite side VA to form basilar artery.[4,5]

Several congenital and structural factors may lead to a reduction in blood flow in the VA, causing ischemia and consequent neurological symptoms.[6] Syncope is the clinical manifestation in the posterior cerebral occlusion or temporary sludge flow in the VA. VA stenosis is the primary risk factor for the recurrent stroke.[7]

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Traditionally, vertebral artery dominance (VAD) is considered a normal congenital variation of VA. It refers to the asymmetry of the diameter of the vessel on both the sides. Increased incidence of VAD is associated with posterior circulation ischemic strokes.[8] Dissection of vertebral arteries are increasingly recognized as a cause of transient ischemic attacks and strokes. The annual incidence of spontaneous vertebral artery dissection is 1 to 1.5 per 100000.[9] Congenital variations in the arrangement and size of the VAs are common. Vertebral artery hypoplasia (VAH) is a frequent (with incidence up to 26%) anatomical variation of vertebral arteries which has been reported among healthy individuals without symptoms of vertebrobasilar insufficiency, and because of its high incidence, the clinical relevance of VAH becomes appreciable.[10] Therefore, precise knowledge of anatomy and variations in the vertebral artery is mandatory while performing angiography, cervical transforaminal epidural steroid injections and fixation and stabilization procedures in cervical spine injuries.

Aims and Objectives

The aim of this research work was to study and compare the different histo-morphometric parameters such as length, inner and outer diameters, width of tunica intima and width of tunica media of 1st segment of vertebral artery on both the sides in the cadavers.

MATERIALS AND METHODS

An institutional cross-sectional study was carried out in Department of Human Anatomy, Peoples College of Medical Sciences & Research Centre, Bhopal over the duration of 3 years with study sample being 40 vertebral arteries obtained from 20 adult human male and female formalin embalmed cadavers.

Inclusion Criteria: Embalmed cadavers (age - 18 years and above)

Exclusion Criteria: Cadavers aged below 18 years, bone deformities at cervical segment, vertebral malformations identified during dissection, fractures of transverse processes of cervical vertebra which may lead to distortion or tearing of vessel wall were all excluded from study.

Ethical Considerations: Necessary approval was taken from the Research Advisory Committee (RAC) and Institutional Human Ethics Committee (IHEC) before proceeding with the research work.

The study included meticulous and systematic dissection of the vertebral arteries of both the sides and staining of the tissues obtained from its first segment as per the protocol. The cadavers were obtained after the MBBS first year undergraduate students had finished the dissection of thorax, face, triangles of neck and deep dissection of neck. Each vertebral artery was identified in the root of the neck arising from the posterosuperior aspect of first part of subclavian artery. On both the sides, V1 of vertebral artery was dissected throughout its length and was made free of its loose connective tissue attachments. The 1st part of vertebral artery was cleared superiorly as it courses between the scalenus anterior and longus colli muscles in front of the transverse process of 7th cervical vertebra. The artery was traced until it passed into the foramen transversarium of the 6th cervical vertebra.[11]

Morphometry of vertebral artery: Following parameters were analyzed and noted: - Site of origin, the point of entry into foramen transversarium of cervical vertebra, any abnormalities in the origin and entry level. Length (L) of 1st segment was measured using white thread and Vernier Calipers in millimeters.

Histomorphometry of Vertebral artery: After identification of first part of vertebral artery, 1cm long tissue above its origin from subclavian artery from both sides was cut out for histological tissue processing and morphometry. After tissue dissection, each cut segment was stored in a neat, clean, sterile separate containers containing 10% formalin solution. The amount of 10% formalin used was about 15-20 times of the tissue. Each container was labelled properly manually with unique identification code. The tissue containers were stored at room temperature till further tissue processing was carried out. Each specimen was studied carefully to note down the gross descriptions like consistency and color of the tissue, presence of blood clot or any other foreign material. Any abnormal deposition inside the lumen was also studied. Furthermore, the tissue specimen obtained was subjected to routine histological tissue processing and staining with Hematoxylin & Eosin staining. The stained slides were viewed using binocular light microscope and high-resolution images were captured using "Motic dedicated Scientific 2 Megapixel Digital Camera Model Moticam A2". Following parameters were measured on the histological slides by using the image analysis software "Motic Images Plus software version 3.0":

D1: Inner lumen diameter of each segment was measured from one end of luminal surface of endothelium of tunica intima to another end of luminal surface of endothelium.

D2: Inner lumen diameter of each segment was measured perpendicular to D1.

Dav: Average inner lumen diameter was calculated using the formula (D1+D2)/2

Ti: Tunica intima thickness measured from endothelial surface to the internal elastic lamina

Tm: Tunica media width measured from internal elastic lamina to external elastic lamina

Do: Outer diameter of the vessel wall was measured using the formula:

Do = Dav + 2Ti + 2Tm 1

Complete set of values obtained from histomorphometry were put on the Ms- Excel worksheet and then the following statistical analysis were applied using SPSS version 29. Mean and standard deviation (SD) and comparison of

ISSN (O): 2687-5365; ISSN (P): 2753-6556

parameters between right and left side was done by using Paired T- test.

RESULTS

In the present study, the first part of vertebral artery originated from the postero-superior aspect of subclavian artery on both the sides in 40 cases (100%). No anomalous origin was observed. In all the 40 (100%) vertebral arteries, V1 segment entered into the foramen transversarium of sixth cervical vertebra. No variation in entry level was observed in the present study. The mean length of V1 segment on right side was observed to be 42.52mm and on left side it was found to be 42.64mm. The mean outer diameter of V1 segment on right side was found to be 2.84mm and on left side it was found to be 3.21mm. The mean inner diameter of the vessel on right side was observed to be 2.63mm and left side it was 2.93mm. The mean thickness of tunica intima on right side was 30.01µm and on left side was 31.01µm. The mean thickness of tunica media on right and left side was observed to be 68.87µm and 72.56µm, respectively. Statistically significant differences were observed in outer and inner diameter of V1 segments on right and left sides.

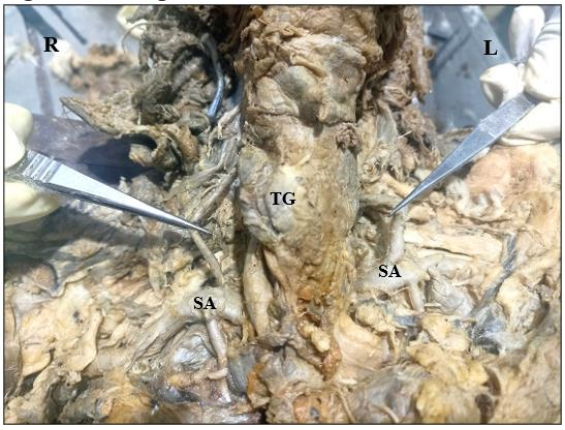

Figure 1: 1st part of Vertebral artery on right and left side (SA – Subclavian artery, TG – Thyroid gland, R – Right side, L – Left side)

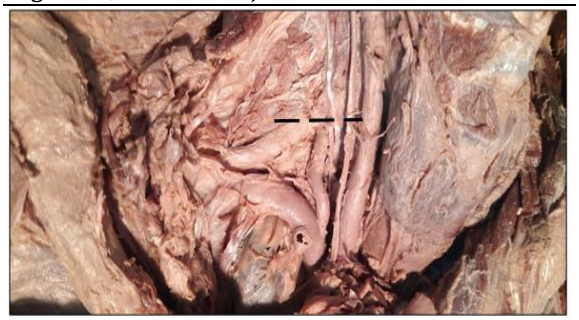

Figure 2: Entry of V1 segment into Foramen transversarium of sixth cervical vertebra on right side

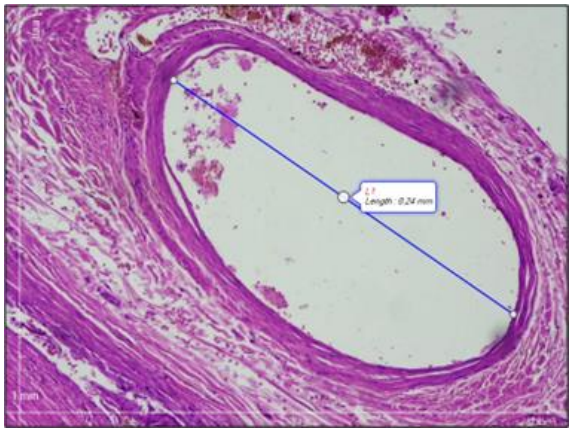

Figure 3: Measurement of inner diameter D1 of vertebral artery (H& E stain – 10x magnification)

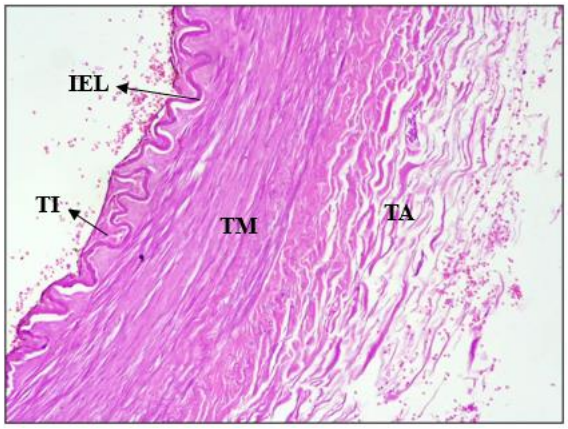

Figure 4: Microscopic structure of V1 segment wherein all three layers of arterial wall can be well appreciated – TI: Tunica intima, IEL: Internal elastic lamina, TM: Tunica media, TA: Tunica adventitia (H&E stain – 10x magnification)

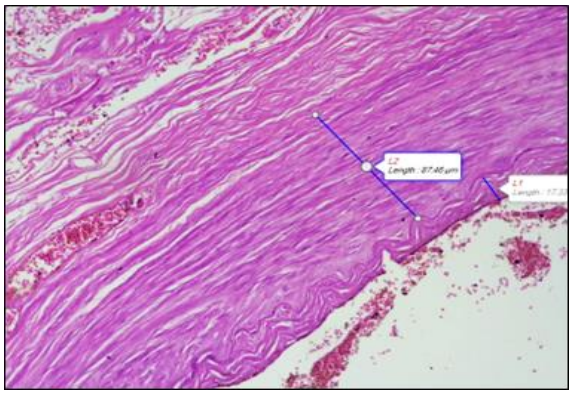

Figure 5: Measurement of thickness of tunica intima (Ti) and tunica media (Tm) of V1 segment (H&E stain – 10x magnification)

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Table 1: Comparison of histomorphometric parameters of V1 segment of right and left vertebral arteries (p value <0.05 is considered statistically significant)

Parameters	Mean±SD		p value
	Rt.	Lt.	
Length	42.52±5.41	42.64±4.89	0.92
Outer diameter	2.84±0.60	3.21±0.63	0.00*
Inner diameter	2.63±0.52	2.93±0.43	0.00*
Width of tunica intima	30.01±9.93	31.01±8.02	0.62
Width of tunica media	68.87±12.85	72.56±14.44	0.23

DISCUSSION

The blood flow in the arterial channels is structured as per the basic and essential laws of fluid dynamics. One should have a thorough knowledge of these laws to develop a better concept and understanding of hemodynamics and physiological phenomenon related to vascular pathologies. The arterial pressure is dependent on the elasticity of its wall and the volume of blood contained in it. The circumferential stress applied to an arterial wall is governed by inner diameter of the vessel wall and its wall thickness. Therefore, a detailed study of histomorphometric parameters of the vertebral artery is necessitated to better understand the hemodynamics within it.[12]

In the present study, statistically significant differences were not observed in the length of V1 on right and left side. However, in the present study, left V1 segment was slightly longer than the right side. A cadaveric study done by Prabavathy in Chennai reported the length of V1 segment on right side to be 50.29±1.48 in males and 40.33±0.67 in females and on left side, 50.02±1.53 in males and 30.68±1.38 in females.[13]

The diameter of vertebral arteries is described in the range of 0.5mm to 5.5mm by Andrew L Carney.[14] Mitchell J described inner and outer diameter of suboccipital and intracranial segment of the vertebral arteries of left and right side, studied in 40 paired suboccipital and 54 paired intracranial vertebral arteries. In his study, it was found that between suboccipital and intracranial part, there was significant difference in diameter ($p \leq 0.001$) but between right and left side, there was no significant difference was found.[12] Diameter of vertebral artery was also measured grossly in 57 human cadavers by Barbara Cagnie et al where diameter was determined by measuring width of the artery and dividing width by 3.14. In their study, mean diameter of left vertebral artery was found to be 3.79±0.80, while right sided diameter was 3.06±1.25 and the differences between the right and left arteries were statistically significant.[15] Vertebral artery diameters were also taken by real time ultra sound measurements and their dimensions were described in different parts of vertebral artery are described by Mitchell J et al.[16]

In the present study, the outer diameter of first segment of right vertebral artery was found to be 2.84±0.60 and on left side was observed to be 3.21±0.63. When paired t test was employed, this difference in the outer diameter on right and left side was found to be statistically significant. In a study done by Sureka et al, a mean outer diameter on right side was 3.8±0.9 and on left vertebral artery was reported to be 3.5±0.9.[17] There is certain amount of shrinkage in tissues in cadavers, which may account for lower values than those reported by real time ultrasound. Asymmetry of the vertebral arteries, with a larger left than right vessel has been described in anatomical books by Cunningham, Mitchell J and McKay A and Machhi et al.[18,19]

In the present study, inner diameter of V1 segment on the right side was reported to be 2.63±0.52 and on the left side it was observed to be 2.93±0.43. The difference in the mean values of inner diameter on right and left side was observed to be statistically significant. The similar findings were observed by Rawal and Jadav wherein they reported a higher luminal diameter on left side (2.74mm) than on the right side (2.64mm). This difference was found to be statistically significant.[1] Desai and Chavan studied inner diameter in 30 vertebral arteries and reported the mean values to be 3.26mm on right side and 3.64mm on left side. They also observed a statistically significant difference in inner diameter on right and left side.[7] Higher inner diameter on left sided vertebral artery in its pre-transverse segment has also been described by Bhadkaria et al.[20] Thus, in the present study, left VA was observed to be dominant than the right VA which is consistent with the previously published literatures.

In the present study, the width of tunica intima on right side was found to be 30.01±9.93 and on the left side, it was observed to be 31.01±8.02. However, the difference on right and left side tunica media was not found to be statistically significant. In study conducted by Rawal et al. in 2017, the tunica intima width of V1 segment was found to be 0.023±0.06.[4] Use of different image analysis software can be held accountable for variable observations in the V1s segment. The tunica intima in the present study was observed to be detached from underlying tunica media. Faulty preservation or tissue damage during histological processing can be the reason behind this finding. Variable thickness of subendothelial connective tissue was observed in all the specimens. In the present study, the width of tunica media was reported to be 68µm and 72µm on right and left side, respectively. Johnson et al reported the width of tunica media to be 0.25mm in V1.[21]

ISSN (O): 2687-5365; ISSN (P): 2753-6556

CONCLUSION

The length of V1 segment on right side was 42.51±5.41 and on left side was 42.64±4.89, the difference was not statistically significant. The outer diameter on right side was observed to be 2.84±0.60 and on left side, it was 3.21±0.63, the difference was statistically significant. Statistically significant differences were also observed in inner diameter on right (2.63mm) and left side (2.93mm). Width of tunica intima was observed to be 30.01µm on right side and 31.01µm on left side. Width of tunica media was found to be 68.87µm and 72.56µm on right and left side, respectively. There are many similarities in the present study and previous studies, although the sample size studied and methodology used were different. The present study can be extended further to study and understand the microscopic anatomical differences in various segments of the vessel and observe the differences in male and female subjects.

Acknowledgement: We thank the Department of Anatomy, PCMS&RC, Peoples University Bhopal (Madhya Pradesh, India) for providing general support to conduct this study.

REFERENCES

1. Rawal JD, Jadav HR. Histomorphometric comparison of diameter of right and left vertebral arteries. Nat J Anat Res. 2012;2(3):260-3.
2. Standring S. Gray's anatomy: The Anatomical basis of clinical practice. In: Standring S, Neil RB, Patricia C, editors. Neck. 40th ed. Edinburg: Churchill Livingstone Elsevier; 2008. p. 449.
3. Rawal JD, Jadav HR. Anatomical study of vertebral artery entering the foramen transversarium of cervical vertebrae. Nat J Anat Res. 2012;2(2):199-201.
4. Standring S. Gray's Anatomy: The Anatomical Basis of Clinical Practice. In: Crossman AR, Collins P, Healy JC, editors. Vascular supply of brain. 37th ed. Edinburg: Elsevier; 2008. p. 250-1.
5. Romanes GJ. Cunningham's Manual of Practical Anatomy. In: Romanes GJ, editors. The prevertebral region. 15th ed. New York: Oxford University Press; 2005. p. 94-5.
6. Ham AW, Cormack DH. Histology. In: Ham AW, Cormack DH, editors. The circulatory system. 8th ed. Pennysylvania: Lippincott William and Wilkins; 1979. p. 593.
7. Desai AR, Chavan SK. Histo-morphometric study of various segments of vertebral artery in human cadavers. Int J Anat Res. 2019;7(1.1):6102-7.
8. Sun Y, Shi YM, Xu P. The clinical research progress of vertebral artery dominance and posterior circulation ischemic stroke. Cerebrovasc Dis. 2022:1-4.
9. Redekop GJ. Extracranial carotid and vertebral artery dissection: A review. Can J Neurol Sci. 2008:35:146-52.
10. Thierfelder KM, Baumann AB, Sommer WH, Armbruster M, Opherk C, Janssen H, et al. Vertebral artery hypoplasia: Frequency and effect on cerebellar blood flow characteristics. Stroke, 2014:1363-8.
11. Romanes GJ. Cunningham's manual of practical anatomy. In: Romanes GJ, editors. The cavity of the thorax. 15th ed. New York: Oxford University Press. 1986. p. 57.
12. Mitchell J. Changes in vertebral artery blood flow following rotation of the cervical spine. Journal of Manipulative and Physiological Therapeutics, 2003; 26: 347-351.
13. Prabavathy G, Sadeesh T, Arthi G, Anbalagn J. Histomorphometry of pretransverse segment of vertebral artery and its clinical significance – a cadaveric study. Int J Curr Res Rev. February 2021;13(4):65-9.
14. Carney AL. Vertebral Artery Surgery: Historical Development, Basic Concepts of Brain Hemodynamics, and Clinical Experience of 102 Cases. In: Carney AL, Anderson EM, editors. Advances in Neurology: Diagnosis and Treatment of Brain Ischemia. New York: Raven Press, 1981; vol.30: 253.
15. Cagnie B, Barbaix E, Vinck E, D'Herde k, Cambier D. Atherosclerosis in the vertebral artery: an intrinsic risk factor in the use of spinal manipulation?.Surg Radiol Anat, 2006; 28: 129 -34.
16. Mitchell J, Kramschuster K. Real-time ultrasound measurements of changes in suboccipital vertebral artery diameter and blood flow velocity associated with cervical spine rotation. Physiother Res Int, 2008; 13(4): 241-54.
17. Sureka B, Mittal MK, Mittal A, Agrawal MSK, Bhambri NK, Thukral BB. Morphometric analysis of diameter and relationship of vertebral artery with respect to transverse foramen in Indian population. Indian J Radiol Imaging. 2015 Apr;25(2):167-72.
18. Mitchell J, McKay A. Comparison of left and right vertebral artery intra-cranial diameters. Anatomical Rec. 1995; 242: 1350-4.
19. Macchi C, Giannelli F, Cecchi F, Gulisano M, Pacini P, Corcos L, Catini C, Brizzi E. The inner diameter of human intra-cranial vertebral artery by color Doppler method. Italian J of Anat Embryol. 1996; 10l: 81-87.
20. Bhadkaria V, Chawre HK, Joshi SS, Joshi SD. Histological variations in various segments of vertebral artery. J Evol Med Dent Sci. 2016;5(02):120–6.
21. Johnson CP, Baugh R, Wilson CA, et al. Age-related changes in the tunica media of the VA: implications for the assessment of vessels injured by trauma. J Clin Pathol. 2001;54:139-45.

ISSN (O): 2687-5365; ISSN (P): 2753-6556